

George N. Serbedzija et al.
Application No.: 09/255,397
Page 2

PATENT
OFFICIAL

- 1 2. (As filed) The method of claim 1, wherein angiogenesis activity is decreased.
- 1 3. (As filed) The method of claim 1, wherein angiogenesis activity is increased.
- 1 4. (As filed) The method of claim 2, wherein the response is a decrease in normal blood
2 vessel formation.
- 1 5. (As filed) The method of claim 3, wherein the response is an increase in normal blood
2 vessel formation.
- 1 6. (As filed) The method of claim 2, wherein the response is loss of existing blood
2 vessels.
- 1 7. (As filed) The method of claim 1, wherein the teleost is an embryo, larva, or adult.
- 1 8. (As filed) The method of claim 1, wherein the teleost is a zebrafish, medaka, Giant
2 rerio, or puffer fish.
- 1 9. (As filed) The method of claim 8, wherein the teleost is a zebrafish embryo.
- 1 10. (As filed) The method of claim 1, wherein the teleost is a wildtype strain.
- 1 11. (As filed) The method of claim 1, wherein the teleost contains a mutation in a selected
2 gene.
- 1 12. (As filed) The method of claim 1, wherein the teleost is transgenic.
- 1 13. (As filed) The method of claim 1, wherein the agent is administered to the teleost by
2 dissolving the agent in media containing the teleost.
- 1 14. (As filed) The method of claim 1, wherein the agent is administered to the teleost by
2 injecting the agent into the teleost.
- 1 15. (As filed) The method of claim 1, wherein the agent is administered to the teleost in
2 conjunction with a carrier.
- 1 16. (As filed) The method of claim 15, wherein the carrier is a solvent, lipid, or peptide.
- 1 17. (As filed) The method of claim 1, wherein the agent is a compound and a library of
2 compounds is screened for angiogenesis activity.

George N. Serbedzija et al.
Application No.: 09/255,397
Page 3

OFFICIAL
PATENT

1 18. (As filed) The method of claim 1, wherein the agent is a nucleic acid, peptide, protein,
2 glycoprotein, carbohydrate, lipid, or glycolipid.

1 19. (As filed) The method of claim 18, wherein the nucleic acid is DNA or RNA.

1 20. (As filed) The method of claim 5, wherein blood vessels are visualized by light
2 microscopy after alkaline phosphatase staining of the teleost.

B1
1 21. (Amended) A method of screening an agent for an effect on cell death
2 activity, said method comprising contacting a living teleost post 12-hours of development with
3 a dye with affinity for dead cells, and thereafter administering the agent to be screened to [a]
4 the living teleost and detecting [a response], the dye in at least one specific tissue or organ in
5 the living teleost indicating an effect on cell death activity in at least one specific tissue or
6 organ of the living teleost.

B2
1 43. (Amended) A method of screening an agent for toxic activity in vivo
2 comprising administering the agent to a teleost in vivo and detecting a change in level of an
3 enzyme or mRNA in at least one tissue or organ of the teleost responsive to the agent [response
4 in the teleost] indicating toxic activity in the at least one tissue or organ of the teleost.

Please add the following new claims:

B3
1 54. (New) The method of claim 21, wherein the response is an increase in
2 cell death activity.

1 55. (New) The method of claim 21, wherein the response is a decrease in
2 cell death activity.

1 56. (New) The method of claim 21, wherein the response is an increase in
2 apoptotic activity or necrotic activity.

1 57. (New) The method of claim 21, wherein the response is a decrease in
2 apoptotic activity or necrotic activity.

George N. Serbedzija et al.
Application No.: 09/255,397
Page 4

PATENT
OFFICIAL

1 58. (New) The method of claim 56, wherein the increase in apoptotic
2 activity comprises an increase in cell death in a tissue or organ of the teleost.

1 59. (New) The method of claim 57, wherein the decrease in apoptotic
2 activity comprises a decrease in cell death in a tissue or organ of the teleost.

1 60. (New) The method of claim 54, wherein the method further comprises
2 detecting a response in cell death activity in the teleost after a predetermined period of time,
3 said time being sufficient for detectable differences in cell death activity to occur in the
4 presence of the agent.

1 61. (New) The method of claim 56, wherein the increase in apoptotic
2 activity is detected by light microscopy or fluorescence microscopy.

1 62. (New) The method of claim 21, wherein the agent is administered to the
2 teleost by dissolving the agent in media containing the teleost.

1 63. (New) The method of claim 21, wherein a fluorescent dye which labels
2 dead or dying cells is administered to the teleost prior to administration of the agent to the
3 teleost.

1 64. (New) The method of claim 63, wherein the fluorescent dye is
2 administered to the teleost by dissolving the fluorescent dye in media containing the teleost.

1 65. (New) The method of claim 63, wherein the fluorescent dye is
2 administered to the teleost by injecting the fluorescent dye into the teleost.

1 66. (New) The method of claim 63, further comprising administering the
2 agent to the teleost by dissolving the agent in the media containing the teleost or injecting the
3 agent into the teleost after administration of the fluorescent dye to the teleost.

1 67. (New) The method of claim 63, wherein a fluorescent dye is a
2 monomeric cyanine dye.

George N. Serbedzija et al.
Application No.: 09/255,397
Page 5

OFFICIAL
PATENT

1 68. (New) The method of claim 67, wherein the fluorescent dye is
2 benzothiazolium-4-quinolium dye.

1 69. (New) The method of claim 21, wherein the teleost is a zebrafish.

1 70. (New) The method of claim 54, wherein the increase in cell death
2 activity is detected in more than one tissue or organ of the teleost simultaneously.

1 71. (New) The method of claim 70, wherein the increase in cell death
2 activity is detected in more than one tissue or organ of the teleost simultaneously over time at
3 predetermined intervals.

1 72. (New) The method of claim 60, wherein the method further comprises
2 detecting the increase in cell death activity over time at predetermined intervals.

1 73. (New) The method of claim 56, wherein the increase in apoptotic
2 activity or necrotic activity is detected in at least one organ or tissue or combination thereof.

1 74. (New) The method of claim 21, wherein the agent is a compound and a
2 library of compounds is screened for an effect on cell death activity.

1 75. (New) The method of claim 43, wherein the response in the teleost
2 indicating toxic activity is detected over time.

1 76. (New) The method of claim 43, wherein the response in the teleost
2 indicating toxic activity is detected in at least two tissues, at least two organs, or at least one
3 tissue and one organ simultaneously.

1 77. (New) The method of claim 76, wherein the response in the teleost
2 indicating toxic activity is over time at predetermined intervals.

1 78. (New) The method of claim 43, further comprising administering the
2 agent to at least two teleosts and detecting a response indicating toxic activity in each of said at
3 least two teleosts simultaneously.

George N. Serbedzija et al.
Application No.: 09/255,397
Page 6

OFFICIAL
PATENT

1 79. (New) The method of claim 78, wherein each of said at least two
2 teleosts is contained in a well of a multi-well plate.

1 80. (New) The method of claim 1, further comprising screening the agent
2 for toxic activity by detecting a response in the teleost indicating toxic activity.

1 81. (New) The method of claim 1, further comprising screening the agent
2 for an ability to enhance or inhibit cell death activity by detecting a response in the teleost
3 indicating an enhancement or inhibition of cell death activity.

1 82. (New) The method of claim 21, further comprising screening the agent
2 for toxic activity by detecting a response in the teleost indicating toxic activity.

1 83. (New) The method of claim 1, wherein the teleost is bleached after
2 staining with alkaline phosphatase.

1 84. (New) The method of claim 21, wherein the method is conducted in a
2 teleost *in vivo*.

1 85. (New) The method of claim 1, wherein the teleost is contained in a
2 microtiter well.

1 86. (New) The method of claim 85, wherein the response is detected using a
2 microplate reader.

1 87. (New) The method of claim 21, wherein the teleost is contained in a
2 microtiter well.

1 88. (New) The method of claim 87, wherein the dye in at least one specific
2 tissue or organ is detected using a microplate reader.

1 89. (New) The method of claim 43, wherein the teleost is contained in a
2 microtiter well.